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## Distribution of *Idanthyrus cretus* (Polychaeta: Sabellariidae) in the Tropical Eastern Pacific and application of PCR-RAPD for population analysis

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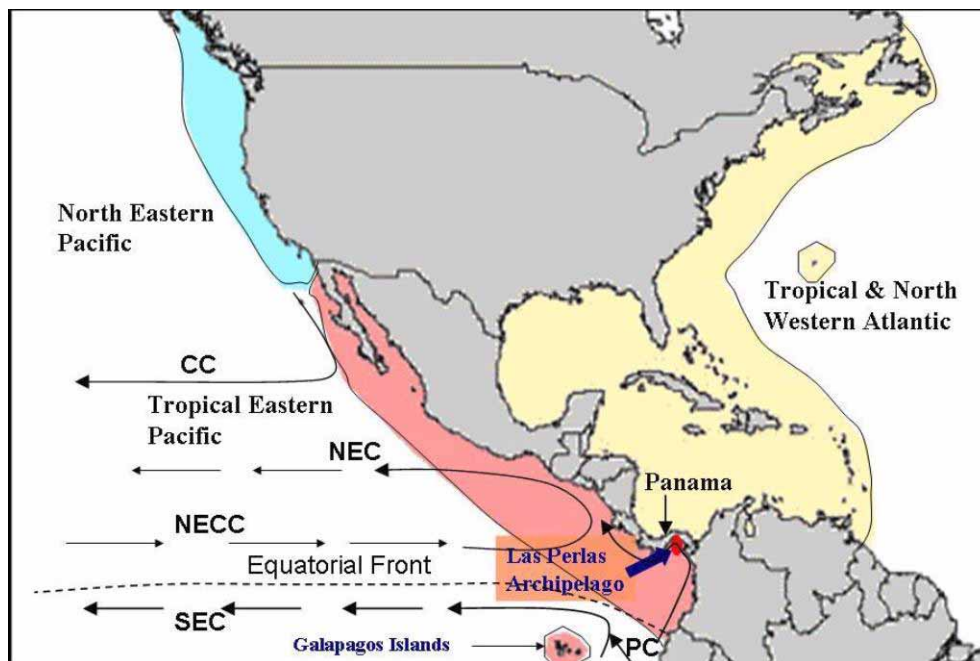
### Abstract

Sabellariid polychaetes, commonly known as honeycomb worms, are capable of forming large reef-like aggregations. One species, *Idanthyrus cretus*, has recently been found in several localities during surveys of Las Perlas Archipelago, Pacific Panama. Large reefs of this species have not been recorded elsewhere in the Tropical Eastern Pacific. The reef distribution patterns were examined and mapped with the aid of Geographical Information Systems. Principal Component Analysis showed that the distribution of polychaete reefs in Las Perlas Archipelago and the reef building itself seem to be mainly dependent on depth, suitable substrate, and topography, the potential particle size for tube building, and wave exposure. Cluster analysis of Random Amplified Polymorphic DNA with Polymerase Chain Reaction molecular techniques demonstrated that the polychaete reefs in Las Perlas Archipelago may be derived from one population.

**Key words:** Polychaeta, Sabellariidae, distribution patterns, Tropical Eastern Pacific, population analysis, molecular techniques

### Introduction

Sabellariid polychaetes, commonly known as honeycomb worms, form large reef-like aggregations in many parts of the world, including the Tropical Eastern Pacific (TEP). Since the first records in 1904 (Chamberlain 1919), many specimens of *Idanthyrus cretus* have been collected from the Pacific coast of Colombia (Hartman 1940), Costa Rica (Hartman 1944), the Galapagos Islands (Hartman 1944; Kirtley 1994), Ecuador (Hartman 1944), and other localities in Panama (Monro 1933; Fauchald 1977; Kirtley 1994). These sites are all in the surf zone (Fig. 1) where the cool (18°–22°C) Peru Current (PC) initially joins the warmer South Equatorial Current (SEC), and then moves westward toward the equator. The PC and the trade winds from the NE produce a coastal upwelling that brings nutrient-rich sea water (Glynn & Mate 1997). The turbidity is caused, therefore, by high nutrient waters that promote the phytoplankton blooms during the dry season (upwelling) and by the sediments released (siltation) with freshwater discharges from rivers due to erosion of adjacent land masses during the wet season (Macintyre et al. 1992, D'Croz & Robertson 1997).



**FIGURE 1.** The Tropical Eastern Pacific and the study area, the Las Perlas Archipelago. CC: California Current, NEC: North Equatorial Current, NECC: North Equatorial countercurrent, SEC: South Equatorial Current, PC: Peru Current. Modified from Glynn and Mate (1997) and REEF (2004).

Nearly all previously recorded sabellariids from the TEP were incorrectly identified as *Idanthyrsus pennatus* Peters, 1854 or *Idanthyrsus armatus* Kinberg, 1867. *Idanthyrsus cretus* Chamberlain, 1919 was first described from Taboguilla Island (Pacific coast of Panama) and also recorded as *Idanthyrsus regalis* Chamberlain, 1919 from the Galapagos Islands. The two species were synonymized by Kirtley (1994) as *I. cretus*. In that revision of the Sabellariidae, many records were reidentified and described as new species (Kirtley 1994).

Sabellariid distribution is constrained by the availability of stable substrates for the settlement of immature stages and sedentary adult stages (Kirtley 1994). There has been extensive work on *Sabellaria alveolata* Linnaeus, 1767 larvae from British waters. For this species it has been demonstrated that the larvae require gentle water movement and more importantly the presence of other newly settled larvae or other living or dead adults to induce settlement (Wilson 1968). All sabellariids are filter feeders and need a constant food supply, high levels of dissolved oxygen, and suspended sediments or shell fragments for tube building (Wilson 1929; Volvelle 1965). Specimens usually occur in the surf zone in water depths less than 10 m, although a few species such as *Gesaia* sp. and *Phalacrostemma* sp. are known from deep waters (Kirtley 1994). For most Sabellariids there is minimal information relating to depth, temperature, salinity, distribution patterns, reproduction, life stages, microhabitats, associated fauna, and associated habitat details. All published records for *I. cretus* (Table 1) are descriptions of single tubes associated with coral reef environments (e.g., Costa Rica and Colombia) or rocky areas in low- to mid-shore or tide pools (e.g., Panama, Ecuador, Galapagos); however, no sabellariid reefs have been recorded in any of these areas.

The Panamanian TEP (see Fig. 1) is a very productive area due to the existence of upwelling (D'Croz et al. 2003). This upwelling leads to shallow thermoclines and enrichment of the coastal and nearshore waters. However, a remarkable characteristic of the TEP is that concentrations of surface nutrients continuously remain high and appear never to be completely depleted despite the high phytoplankton productivity (Agujetas-Medrano 2004). There is also high turbidity that results in low biodiversity in some taxonomic groups like corals (Glynn & Mate 1997; D'Croz & Robertson 1997;

Diaz et al. 2000) in contrast with the Tropical Western Pacific (Glynn 2001). The upwelling occurs each year during the dry season that lasts from January to April (D'Croz et al. 2001), with the coldest period in March (average 20°C) and the warmest period (average 28°C) between May and July (D'Croz & Robertson 1997; D'Croz & O'Dea 2007).

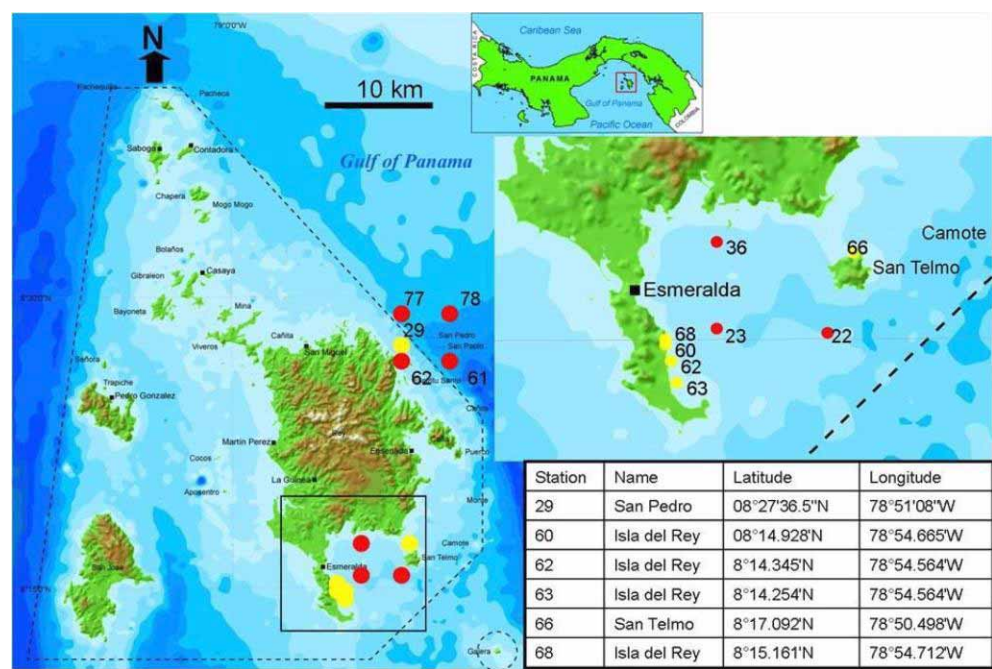
**TABLE 1.** Distribution of *Idanthysus cretus* in the Tropical Eastern Pacific and main habitat details.

Locality	Original identification	Kirtley (1994) identification	Habitat details	Reference
Panama, Isla Taboguilla	<i>I. cretus</i> (holotype)	<i>I. cretus</i>	1.8 m, coral.	Chamberlain 1919: 485
Panama, Taboga Island	<i>I. pennatus</i>	<i>I. cretus</i>	Hotel Pier	Monro 1933: 1065
Panama, Gorgona Island	<i>I. pennatus</i>	<i>I. cretus</i>	From coral at low tide and at 1 fth, in tubes or solitary	Monro 1933: 1065
Panama, Gorgona Island, Taboga Island (Monro's specimen)	<i>I. pennatus</i>	<i>I. cretus</i>	All low to mid shore.	Fauchald 1977: 54
Panama, Pinas Bay, Secas Island, Coiba Island	<i>I. pennatus</i>	<i>I. cretus</i>	3.6–7.3 m tubes overgrown with coral,	Hartman 1944: 336 RV <i>Velero</i>
Panama, Pedro Gonzales Island, Islas Perlas	<i>I. pennatus</i>			Kirtley 1994: 95
Galapagos, Floreana, Tagos Cove, Chatham Island, Sullivan Bay James Island	<i>Idanthysus</i> sp?	<i>I. cretus</i>	Shore and tide pool	Kirtley 1994: 95
<i>Velero</i> exp from Mexico to Ecuador	<i>I. pennatus</i> <i>I. armatus</i>	<i>I. cretus</i>	Shore to 6 m	Hartman 1944: 336, 131
Colombia, Puerto Utria, reefs, Octavia Bay	<i>I. pennatus</i>	<i>I. cretus</i>	Outer island, shore, on <i>Pocillopora</i> coral. All shallow to 10–11 m coarse sand and gravel	Hartman 1944: 336 RV <i>Velero</i>
Costa Rica, Playa Blanca, Viradores Islands, Puerto Culebra	<i>I. pennatus</i>	<i>I. cretus</i>	on <i>Poronia</i> coral	Hartman 1944: 336 RV <i>Velero</i>
Ecuador, Punta Santa Elena, coll. Waldo Schmitt	<i>I. pennatus</i>	<i>I. cretus</i>	tide pool	Hartman 1944: 336 RV <i>Velero</i>
Galapagos, Gardner Bay Hood Is, Flamingo Bay Charles Island, Albemarle Sound, Duncan Island, Darwin Bay Tower Island, Sullivan Bay James Island	<i>I. pennatus</i>	<i>I. cretus</i>	shore	Hartman 1944: 336  RV <i>Velero</i>
Galapagos	<i>I. regalis</i> (holotype)	<i>I. cretus</i>	Chatham Island	Chamberlain 1919: 487

The aim of the present study was to document distribution patterns for *Idanthyrus cretus* in the TEP in relation to physical data. The analysis of the population data of the Las Perlas colonies included a molecular approach by using cluster analysis of Random Amplified Polymorphic DNA with Polymerase Chain Reaction (PCR-RAPD) techniques to investigate possible genetic links and geographic origins.

### Materials and methods

**Field survey (the polychaete reef environment).** Las Perlas Archipelago is an area to the SE of Panama Bay (8°14'00" to 8°39'00" N/ 78°50'00" to 79°06'36" W) and consists of one large, two or three medium, and several small islands (Fig. 2). Basic ecological information (e.g., polychaete reef distribution, associated fauna), collected between August 2004 and May 2005, was obtained from six stations in 2–6 m water depth relative to low tide. Stations 60, 62, 63, 66, and 68 correspond to a sand habitat and station 29 corresponds to a coral reef habitat (Benfield 2005; Barrios 2007). The six stations were selected for detailed study based on data collected during several surveys in the area in 2003 and 2004 (Benfield 2005). Station 66, with the largest reef, was subdivided into seven stations, resulting in a total of 12 stations.



**FIGURE 2.** Field site: Las Perlas Archipelago. Circles in yellow are the six stations where samples and data were collected. Circles in orange are the stations for sediments (22, 23, 36, 61, 62, 77, 78). Courtesy of Sarah Benfield database (Benfield 2005).

At each station, at least 10 organisms were removed by cutting off a section of the tube aggregate (blocks of sand tubes), except at station 29 where only two organisms were collected (they were found individually as part of a reef environment and not as tube aggregates). The samples were fixed in 10% formalin, preserved in 74% alcohol, identified, and deposited at the National Museums of Scotland (Register Number NMSZ.2006.073). Samples fixed and preserved in 96% ethanol were analyzed with molecular techniques to determine their population origin. All species associated with the sections of polychaete reef removed from each station were identified to phylum level.

Information on the density and dimensions of the polychaete reef (to identify polychaete reef structure) and on the surrounding environment (sediment type, type of benthic community, distribution of the species and percent cover in coral reef environments) was also collected by conducting 30-minute visual surveys. At the largest reef (station 66, San Telmo), one length and three width measurements were recorded. One single width and one single length were recorded in smaller aggregates. Density was measured in the larger reef with 1-m<sup>2</sup> quadrats (a total of six quadrats) subdivided into squares of 10 x 10 cm. No measures of density were taken in the smaller reefs as they appeared to have the same development pattern observed in the largest reef.

Substrate information collected during the 2003 and 2004 surveys (Benfield 2005) related to coral reefs (e.g., percent cover of benthic organisms, visibility, topography) was used to describe habitats surrounding the polychaete reefs in San Telmo (station 66) and Isla del Rey (stations 60, 62, 63, 68), and as part of the environmental information for statistical analysis. Sea Surface Temperature–SST and salinity records collected at the Smithsonian Tropical Research Institute–STRI were used for comparison with the data collected during the field surveys. Sediment information collected during the 2003 and 2004 surveys (Benfield 2005) related to percent sediment composition (gravel, very coarse sand, coarse sand, medium sand, fine sand, very fine sand, silt+clay) near the polychaete reef stations (see Fig. 2, stations 22, 23, 36, 61, 62, 77, 78) was used as part of the environmental information for statistical analysis (Table 2).

**TABLE 2.** Type of sediments in areas around the stations (values for sediment particle sizes in percentage, %). Grade limits (µm) in Wentworth grade classification: Gravel (4000–2000), Very Coarse Sand (2000–1000), Coarse Sand (1000–500), Medium Sand (500–250), Fine Sand (250–125), Very Fine Sand (125–62), Silt+Clay (<62).

Nearest polychaete station	Station for sediments	Sediment type	Gravel	Very Coarse Sand	Coarse Sand	Medium Sand	Fine Sand	Very Fine Sand	Silt +Clay
66	22	sand	4.6	17.4	24.9	39.5	13.0	0.2	0.5
68,60,62,63	23	sand + shells	1.3	1.1	1.0	4.2	58.4	22.6	11.5
68,60,62,63	36	mud + sand	4.1	1.6	1.3	1.7	13.0	60.5	17.8
29	61	mud	0.0	0.2	0.1	0.0	8.7	36.3	54.7
29	62	mud	0.2	0.0	0.3	0.9	7.3	38.3	52.9
29	77	mud	0.6	0.2	0.1	0.3	1.1	17.9	79.9
29	78	mud	0.5	1.0	0.6	0.2	1.1	10.3	86.2

**Material examined for confirmation of the species.** The holotypes of *Idanthyrus cretus* Chamberlain, 1919 from Taboguilla Island, Panama Bay (USNM 19716), *I. regalis* Chamberlain, 1919 collected 1 September 1905 from Galapagos Islands (USNM 19383), specimens identified as *I. pennatus* by Monro (1933) from Taboga Island, Panama Bay (NHM.1933.7.10.192-196) and Gorgona Island, Colombia (NHM. 1933.7.10.182-19), and five representative specimens from 50 complete polychaete collected August 2004 in San Telmo Island, Las Perlas Archipelago (NMSZ 2006.073) were examined.

**Molecular analysis.** The molecular analysis was carried out at the Center for Marine Biodiversity and Biotechnology, Heriot-Watt University. Samples of *I. cretus* were collected from all 12 stations, for a total of 46 samples, and prepared for DNA extraction (QIAGEN 2006). The final solutions were kept in a freezer at -20°C.

PCR-RAPD protocols, as described by Soosten et al. (1998) and Schmidt & Westheide (1999), were used with minor modifications for DNA amplification of the 46 samples. From the original protocols a total of 17 decamer primers OPB (www.mwg-biotech.com, Germany) with a GC-content between 60 and 70% (Table 3) were applied. The reaction volume of 25 µl was composed of 12.5 µl of 2 x biomix buffer (Bioline), 0.25 µl of mwg primer, 1 µl DNA, and 11.25 µl DH<sub>2</sub>O (MiliQ). Controls were run changing the concentration of DNA for DH<sub>2</sub>O. During amplification, one main thermocycler was used (BIORAD, Hercules, California). Forty-five cycles were employed (modified from Soosten *et al.* 1998), each comprising:

- Step 1: 94°C/4 min (1 cycle)
- Step 2: 94°C/1 min for denaturation, 34°C/1 min for annealing, and 72°C/30 sec for elongation (fastest available transition between temperatures), (45 cycles)
- Step 3: 72°C/10 min (1 cycle),
- Step 4: endless file at 4°C/ ∞

**TABLE 3.** Decamer primers (www.mwg-biotech.com, Germany) and their Guanine-Cysteine (GC) content.

PRIMER	SEQUENCE 5'-3'	GC content (%)
OPB-1	GTTTCGCTCC	60
OPB-2	TGATCCCTGG	60
OPB-3	CATCCCCCTG	70
OPB-4	GGACTGGAGT	60
OPB-5	TGCGCCCTTC	70
OPB-6	TGCTCTGCCC	70
OPB-7	GGTGACGCAG	70
OPB-8	GTCCACACGC	70
OPB-10	CTGCTGGGAC	70
OPB-11	GTAGACCCGT	60
OPB-12	CCTTGACGCA	60
OPB-14	TCCGCTCTGG	70
OPB-15	GGAGGGTGTT	60
OPB-16	TTTGCCCGGA	60
OPB-17	AGGGAACGAG	60
OPB-18	CCACAGCAGT	60
OPB-19	ACCCCGAAG	70

Amplified DNA fragments were separated with a gel loading dye (Fermentas Company) at 82 volts for 10 min followed by 90 volts at maximum 20–30 min (best running time for gels with eight pools was 25 minutes, until samples reached the middle). The ethidium bromide stain was visualized and documented under UV light. In order to optimize the best running time for the primer screening, bigger gels (16 pools in one line, 1% agarose, SCIEPLAS Company, www.scieplas.co.uk) were run for a maximum of 2.5 h (82 volts for 10 min, followed by 90 volts), with images recorded at 1.5 h, 2 h and 2.5 h (best running time for gels with 16 pools was 2 h). A set of 10,000BP, 1,000BP, and 100BP ladders (Fermentas Company) was used for length comparison during the whole procedure. The results were run in a gel of 1% agarose (TAE 1X).



The reproducibility of the data was checked at regular intervals on different levels throughout parallel experiment lines, including potential differences in the amplification patterns that might be derived from the use of different thermocyclers (both thermocyclers were from the same company) as recommended in Soosten et al. (1998) (i.e., use two different thermocyclers to analyze a group of samples and their replicates, and use two different gels to run a same group of RAPD-PCR products that have been processed in the same thermocycler).

For the primer screening, and in order to have the entire spectrum of possibilities, a combination of all 46 samples was used with the three primers, OPB-5, OPB-6, OPB-18, that gave the best results in the Soosten et al. (1998) and Schmidt & Westheide (1999) analyses. Three samples with the biggest polymorphism expression (largest number of bands) were then selected to compare with the other 14 primers (see Table 2). In each series, one reaction was carried out with a blind sample lacking DNA to check for contamination with foreign DNA.

**Statistical analysis.** To identify the main environmental factors responsible for the *I. cretus* distribution observed at the Las Perlas Archipelago (differences between stations), Principal Component Analysis (PCA) was applied to the data using Minitab software (Wardlaw 2001). The variables included were: depth (meters); percent sediment composition (gravel, very coarse sand, coarse sand, medium sand, fine sand, very fine sand, silt+clay); wave exposure (1 = sheltered e.g., bay, 2 = low to moderate, 3 = moderate, 4 = moderate to high, 5 = high e.g., headland); visibility (meters); percent coral cover with respect to total hard bottom area; topography (1 = no vertical or very low vertical structure or complexity, 2 = low or with undulations from 0 to 0.5 m, 3 = moderate or with 0.5 to 1 m outcrops, some boulders, crevices and overhangs, 4 = high or with 1.5 to >2 m outcrops, many overhangs, crevices and boulders). Due to the fact that the variables were measured on different scales, a standardization (Standard Deviation) was performed before the analysis (correlation of the different variables).

To identify possible genetic links between the organisms from each station and relate these links to a possible common geographic origin, regression and cluster analyses were applied to the results of the molecular analysis using specialized software. The detected amplification product patterns collected from the gels were examined with the GEL-COMPARE software to determine the number of stable bands in common or the number of monomorphic and polymorphic markers (Applied Maths BVBA 1998). In this case, an identification group of the taxonomic unit was created from the results of the gels, containing a collection of representative fingerprints, representing the variability within the group. The software then counted the relative frequency of occurrence for each individual band, assigning more weight to bands with a high occurrence frequency (typical bands). The main regression in each gel (group of bands in a particular gel vs. group of bands in the identification group) was based on the differences in concentration and distance of each band from the origin of the gel. A secondary regression was based on the differences in morphology and/or staining of the band groups vs. the identification group. Cluster analysis was carried out with UPGMA methods (unweighted pair-group method using arithmetic averages), based on the band information provided by the polymorphism analysis. For the analysis the recommended coefficients of similarity were applied (Pearson correlation coefficient for clusters on single gels and averages from experiments for clusters from combinations of gels).

## Results

### The polychaete reef environment

Twelve reefs or aggregates of *I. cretus* were found during the 2003–2004 surveys (Benfield 2005).



They were southeast (SE) of the Las Perlas Archipelago, specifically SE of Isla del Rey and north of San Telmo Island (see Fig. 2). During the 2004–2005 surveys it was evident that the area SE of Isla del Rey is characterized by uncolonized rock surrounded by sand bottoms, while the northern part of San Telmo Island is a coral community surrounded by sand bottoms.

All reefs are exposed to strong currents and wave action from the SE Pacific (which is high in energy and suspended particulates). Five of the polychaete reefs in San Telmo and Isla del Rey were analyzed. Their depth range was 0–6 m and the top of the reef at San Telmo was exposed at low spring tides. The approximate size of the largest *I. cretus* reef (station 66) was 56 m<sup>2</sup> (3.5 m average width x 16 m total length) and had an elongated shape parallel to the coast, but the other four reefs had a rounded shape with sizes between 2 and 4.5 m<sup>2</sup>. No reefs had been recorded on this scale before the survey done in 2003 and 2004 to collect information on the environments surrounding the archipelago. The San Pedro samples (station 29, two organisms) were found amongst corals as single tubes but did not form reefs.

The average density of organisms in the largest *I. cretus* reef (station 66), was 72.2 organisms/10 cm<sup>2</sup>, with a maximum of 116 and a minimum of 49/10 cm<sup>2</sup>. Observations from the field showed that each individual captures food and fragments of rock and shells continuously with the oral filaments; these activities also appear to help in the production of the cylindrical tube. The species grows in aggregates and seems to have sexual and asexual reproduction within the aggregates. Observations of the aggregates, morphological descriptions of the external and internal parts of live and preserved organisms (Figs. 3, 4), and comparisons of these descriptions with those reported previously for another related species such as *Sabellaria alveolata* (Cazaux 1964; Volvelle 1965) indicated a sexual dimorphism in *I. cretus* as there were big dark tubes which could correspond to females, surrounded by smaller ivory tubes that could correspond to males (see Fig. 5). In the case of *S. alveolata*, the sexual dimorphism is expressed in females by pinkish purple sides and in males by whitish sides.

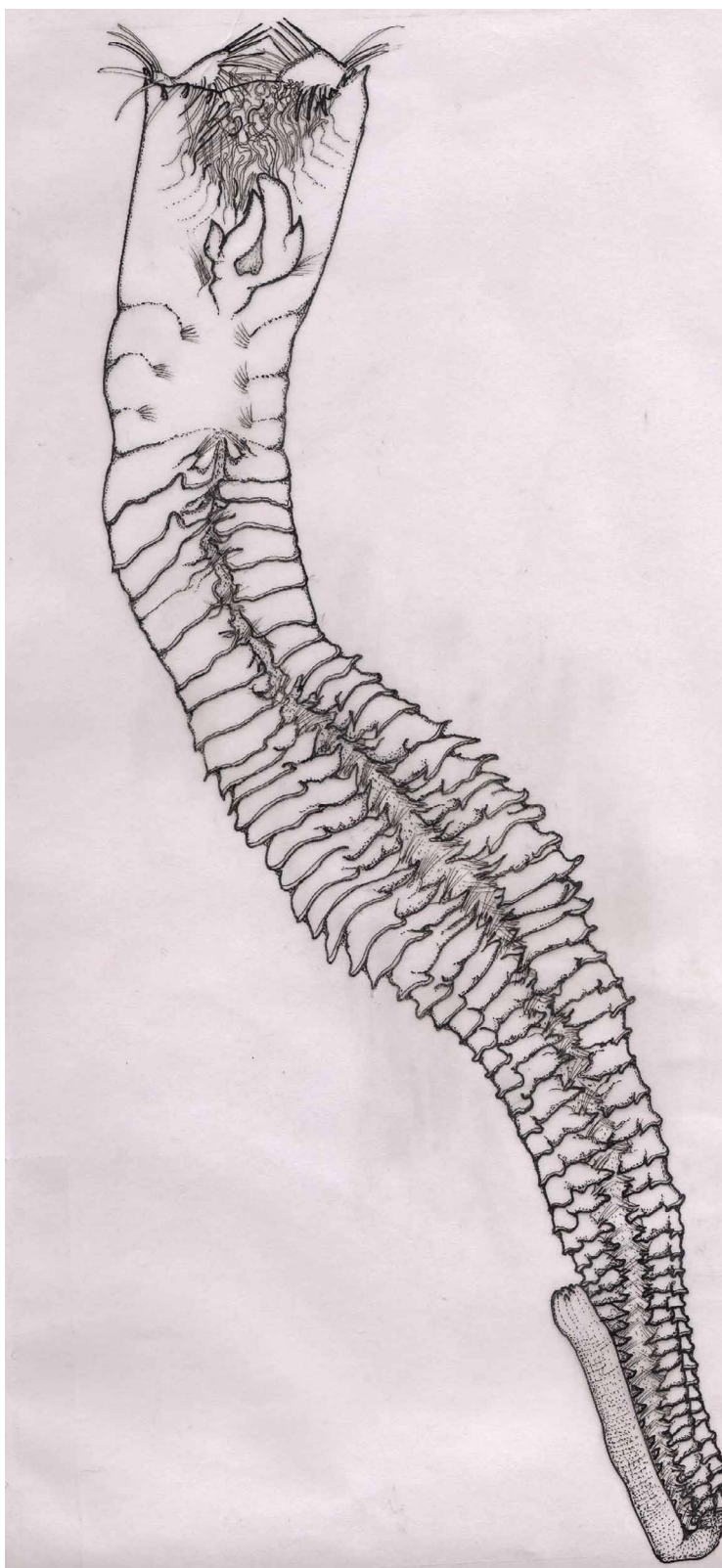
The reefs also provide a habitat for a diverse fauna of sponges, cnidarians, other polychaetes, mollusks, crustaceans, echinoderms, and fish, with an average of seven different phyla per station (Table 4).

The PCA of 12 variables (depth, % gravel, % very coarse sand, % coarse sand, % medium sand, % fine sand, % very fine sand, % silt+clay, wave exposure, visibility, coral cover, topography) showed that two main components are responsible for 100% of the polychaete reef distribution in Las Perlas Archipelago and the differences observed between the environments in which the polychaetes were found. The first principal component, PC1, has a variance (eigen value) of 6.419 and explains 53.5% of the distribution of the aggregations in the area. The second component, PC2, has a variance of 5.581 and explains the other 46.5% of the distribution. The results also demonstrate that PC1 is a combination of % gravel, wave exposure, and topography (and % silt+clay but in a smaller proportion and with a positive value), while PC2 is a combination of depth, fine and very fine sand in the area (as well as visibility and coral cover but in less proportion and with negative value) (Fig. 6).

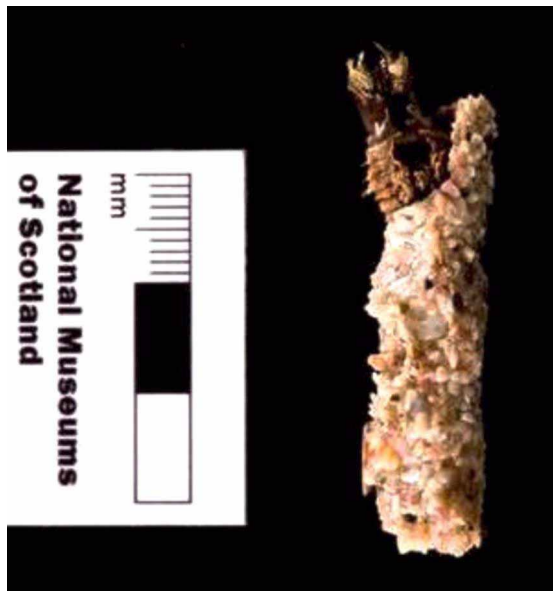
#### Material examined for confirmation of the species

The holotype of *Idanthyrus cretus* Chamberlain, 1919 from Taboguilla Island, Panama Bay (USNM 19716), measured 3 x 10 mm for 23 segments. The holotype of *I. regalis* Chamberlain, 1919 from Galapagos Islands (USNM 19383), measured 5 x 40 mm for 27 segments. The specimens identified as *I. pennatus* by Monro (1933) from Taboga Island, Panama Bay (NHM.1933.7.10.192-196) and Gorgona Island, Colombia (NHM. 1933.7.10.182-19) were all incomplete with no tail region; the specimen from Taboga measured 2 x 14 mm for 35 segments and the specimens from Gorgona 1 x 10 mm for 29 segments and 2 x 13 mm for 31 segments. From the 50 complete specimens collected during the August 2004 survey in the Las Perlas Archipelago (NMSZ 2006.073), 42 were used

partially for molecular purposes and five specimens were used for taxonomic confirmation purposes (see Figs. 3–4). The measurements for the five specimens were: 5 x 41 mm for 53 segments, 5 x 51 mm for 59 segments, 3 x 27 mm for 45 segments, 4 x 21 mm for 44 segments, and 4 x 23 mm for 45 segments.



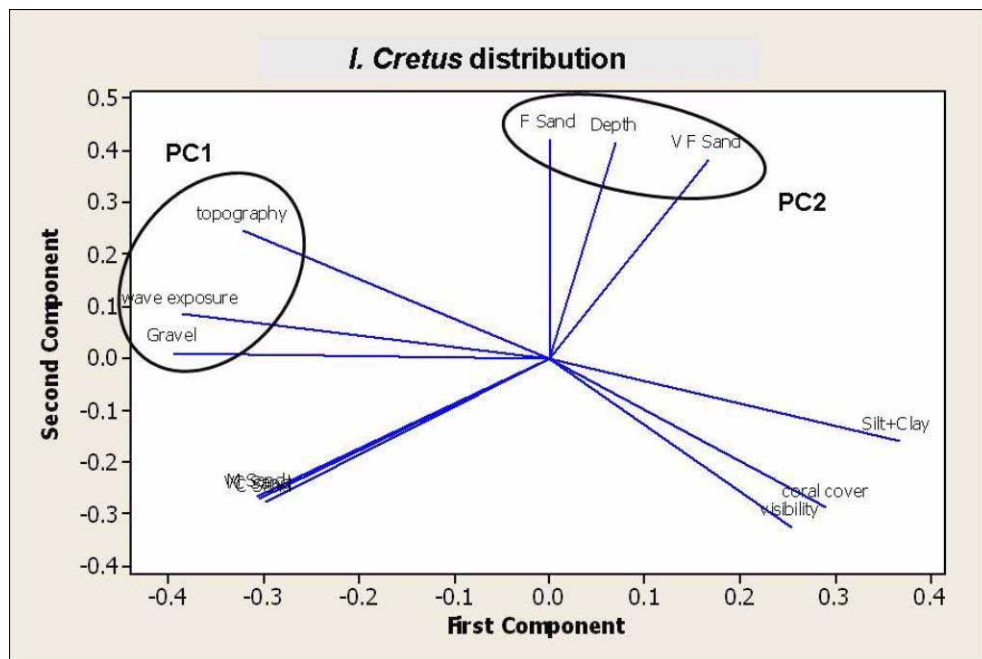
**FIGURE 3.** Ventral view of *Idanthysus cretus* (NMSZ 2006.73) from San Telmo Island. Organism removed from tube measured 41mm.



**FIGURE 4.** *Idanthyrus cretus* (NMSZ 2006.73) from San Telmo Island. Section of tube with animal protruding.



**FIGURE 5.** *I. cretus* section of reef from station 66 in San Telmo Island. The arrows indicate the large female tubes surrounded by small male tubes.



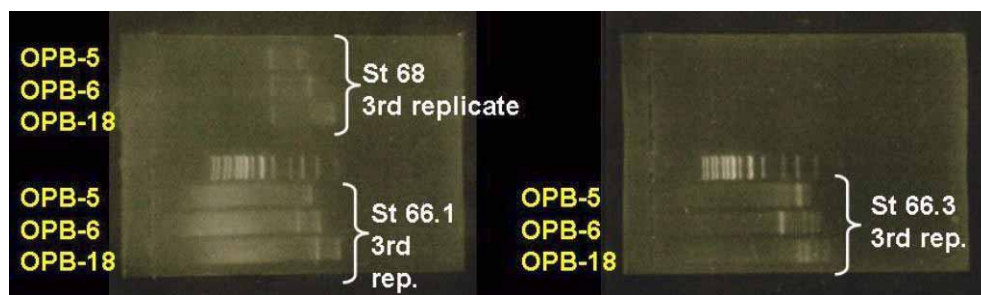
**FIGURE 6.** Principal Component Analysis for the first two components, which explain 100% of the polychaete distribution.

**TABLE 4.** Number of associated species/taxa (total organisms/taxa) in the *Idanthyrus cretus* reef in San Telmo Island. Average species/station = 6.55.

Groups/Station	66.1	66.2	66.3	66.4	66.5	66.6	66.7	66.8	66.9	Total spp/ taxon	Total organisms/ taxon
Porifera								1(1)		1	1
Cnidaria	1(1)	1(1)								2	2
Polychaeta	1(1)			1(1)	5(5)					7	7
Mollusca	1(2)	5(5)		1(1)				3(3)		10	11
Crustacea	7(14)	6(9)	1(1)	4(5)	3(4)		1(1)	3(9)	2(2)	27	45
Echinodermata	2(4)	4(14)	1(2)	1(1)		1(2)	1(1)		1(2)	11	26
Vertebrata		1								1	1
<b>Total spp./station</b>	12	17	2	7	8	1	2	7	3		

### Molecular analysis

The primer screening with OPB-5, OPB-6, and OPB-18 (Fig. 7) demonstrated that Primer OPB-18 was more discriminating than OPB-6 and both revealed more bands or polymorphism than OPB-5. Each station presented a different band pattern when compared with the same primer. Station 68 had five bands with primer OPB-18, while station 66.1 had two bands and station 66.3 between six and seven bands. However, the last two stations from San Telmo (66.1 and 66.3) illustrated only slight variation in the bands. Samples 66.3.3 and 62.2, with the best polymorphism results (Table 5), were selected to run the complete primer screen with all 17 primers, and to identify the best primer for this species (Figs. 8–9).

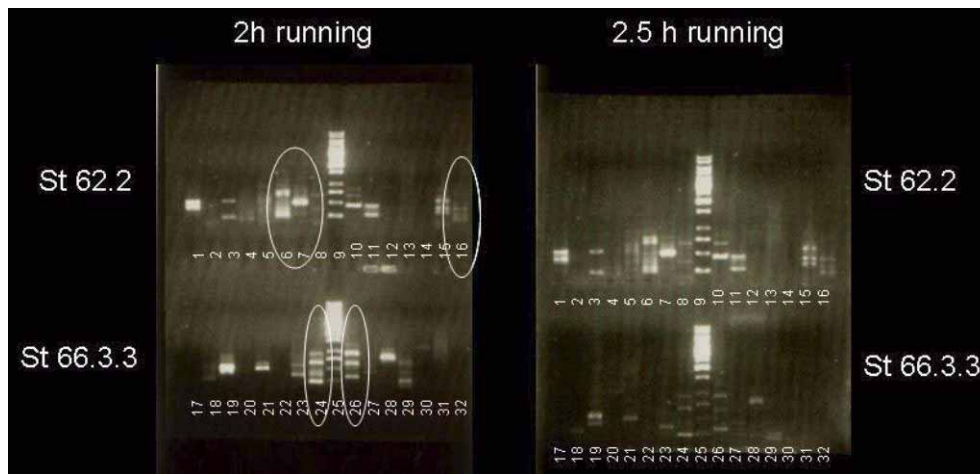


**FIGURE 7.** Results of primer screening with decamer primers OPB-5, OPB-6, OPB-18. Gel 1 (top left) is station 68 from SE Isla del Rey. Bottom left Station 66.1 in San Telmo Island. Gel 2 (right): station 66.3 in San Telmo Island.

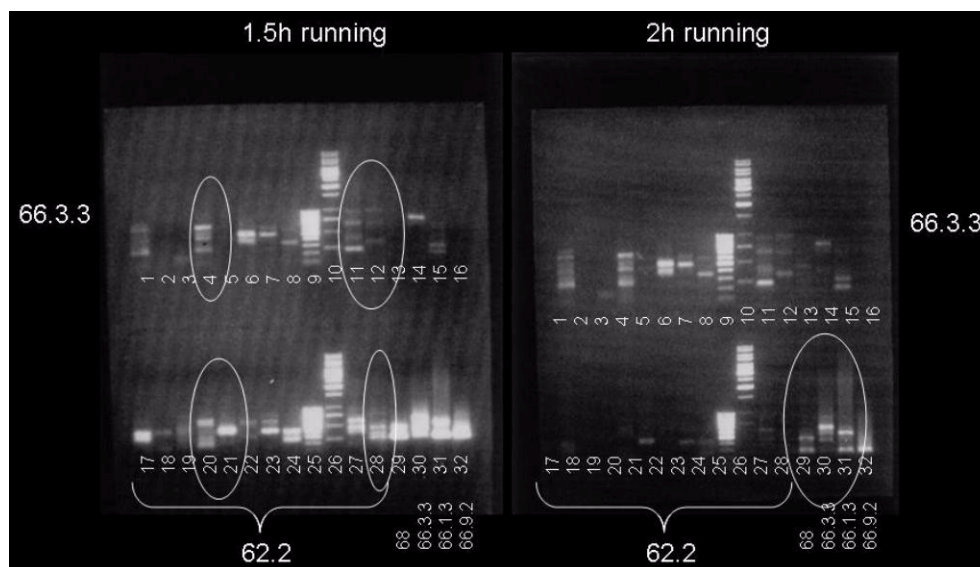
**TABLE 5.** Samples with the largest number of bands (polymorphism) from the analysis with the first three primers: OPB-5, OPB-6, OPB-18. NA: information not available.

Station	Replicate	No. bands OPB-5	No. bands OPB-6	No. bands OPB-18
66.3	3	4	6	6-7
62	2	NA	3(+)	7
68	3	3	2(+)	5(+)
66.8	3	1	1	6
66.5	3	1(+)	1	3
66.4	4	NA	NA	4
60	4	NA	NA	4





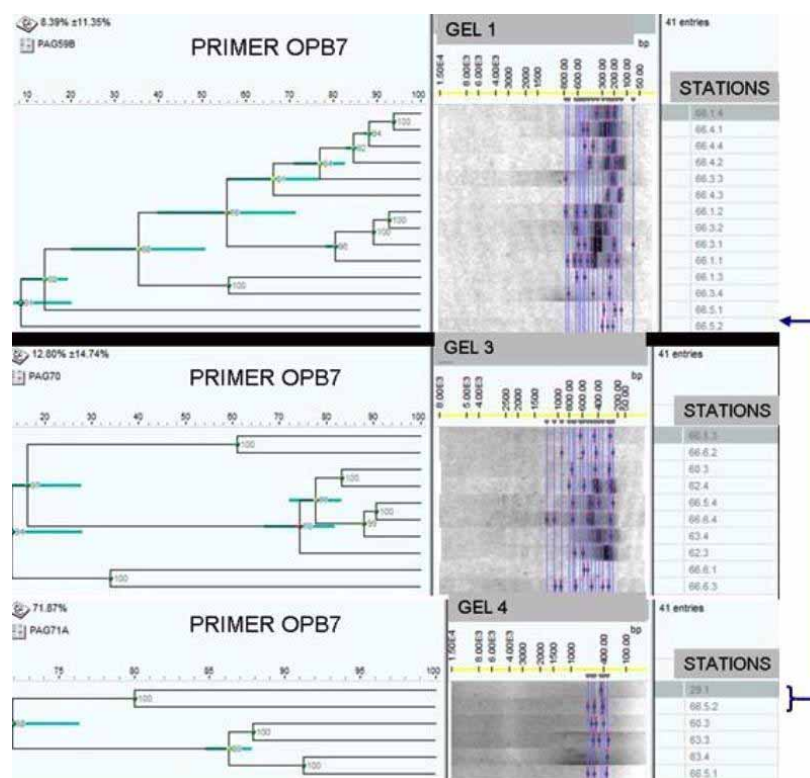
**FIGURE 8.** Primer screen with the two selected samples (station 62, second replicate, station 66.3, third replicate). Left: gel (1% agarose, SCIEPLAS) after 2 hours of electrophoresis. Right: same gel after 2.5 hours. Pools 1 to 16 correspond to analysis with sample 62.2. Pools 17 to 32 correspond to analysis with sample 66.3.3. 1:OPB-1, 2:OPB-2, 3: OPB-3, 4: OPB-4, 5: OPB-6, 6:OPB-7, 7: OPB-8, 8: OPB-10, 9: ladder, 10: OPB-11, 11: OPB-12, 12: OPB-14, 13: OPB-15, 14: OPB-16, 15: OPB-17, 16: OPB-18, 17: OPB-19, 18: control, 19: OPB-1, 20: OPB-2, 21: OPB-3, 22: OPB-4, 23: OPB-6, 24: OPB-7, 25: ladder, 26: OPB-8, 27: OPB-10, 28: OPB-11, 29: OPB-12, 30: OPB-14, 31: OPB-15, 32:OPB-16. Circles highlight pools 6 and 7, 16, and 24 and 26 with the primers OPB-7, OPB-8, and OPB-18 respectively.



**FIGURE 9.** Test of reproducibility. Analysis with all the primers and samples 66.3.3 and 62.2 confirm the results on previous gel (Fig. 8) with the same samples. In this second case OPB-18 (pools 4 and 28), OPB-7 (pools 11 and 20) and OPB-8 (pools 12 and 21) give the best results in terms of bands (polymorphism) and definition. Pools 29 to 32 confirm reproducibility when using different thermocyclers. Samples from pools 29 to 31 are RAPD-PCR products for OPB-18, the same primer and samples than in figure 8, but that were run in a second thermocycler. Pools 1 to 16 are analyses with samples from station 66.3 (third replicate). Pools 17 to 28 are analyses with samples from station 62 (second replicate). 1: control, 2: OPB-16, 3: OPB-17, 4: OPB-18, 5: OPB-19, 6: OPB-1, 7: OPB-3, 8: OPB-6, 9: ladder 100bp, 10: ladder 1kbp, 11: OPB-7, 12: OPB-8, 13: OPB-10, 14: OPB-11, 15: OPB-12, 16: OPB-13, 17: OPB-1, 18: OPB-3, 19: OPB-6, 20: OPB-7, 21: OPB-8, 22: OPB-10, 23: OPB-11, 24: OPB-12, 25: ladder 100bp, 26: ladder 1kbp, 27: OPB-17, 28: OPB-18, 29: St 68 with OPB-18, 30: St 66.3.3 with OPB-18, 31: St 66.1.3 with OPB-18, 32: St 66.9.2 with OPB-18.

The results of the complete primer screen and test of reproducibility (see circles in Figs. 8–9) showed that primers OPB-7, OPB-8, and OPB-18 gave the best polymorphism results and definition or sharpness of the bands, and therefore are the most suitable primers to analyze for this species. The analysis with all the primers and samples 66.3.3 and 62.2 (Fig. 9) confirmed the results on previous gels (Fig. 8) with the same samples. In this second case (see Fig. 9) OPB-18 (pools 4 and 28), OPB-7 (pools 11 and 20), and OPB-8 (pools 12 and 21) gave the best results in terms of bands (polymorphism) and definition. Pools 29–32 were used to confirm reproducibility when using different thermocyclers. In this case, samples from pools 29–31 were RAPD-PCR products for OPB-18, the same primer and samples as in figure 8, but run in a second thermocycler from the same company. Pools 1–16 were analyses with samples from station 66.3 (3<sup>rd</sup> replicate), while pools 17–28 were analyses with samples from station 62 (2<sup>nd</sup> replicate). Fifty (50) DNA fragments were detected with primer OPB-18 and 55 with primer OPB-7 by using band-matching analysis (software GELCOMPARE) for all the samples.

The cluster analyses, run to identify levels of similarity between samples and between stations based on the degree of polymorphism of the samples, showed that there were no statistically significant differences between stations (Fig. 10). Higher similarity between samples belonging to different stations is shown in gel 4 (see Fig. 10). Here, the second sample from the San Telmo station 66.5 (sample 66.5.2, SE of the archipelago) is more related to the first sample from San Pedro station 29 (sample 29.1, NE of Isla del Rey) than to the other sample from San Telmo (sample 66.5.1). In the same way, sample 63.3 is more similar to sample 60.3 than to sample 63.4. The same pattern is evident in gels 1 and 3 (see Fig. 10, arrow for sample 66.5.2 in gel 1 and clustering of samples from station 66 in San Telmo).



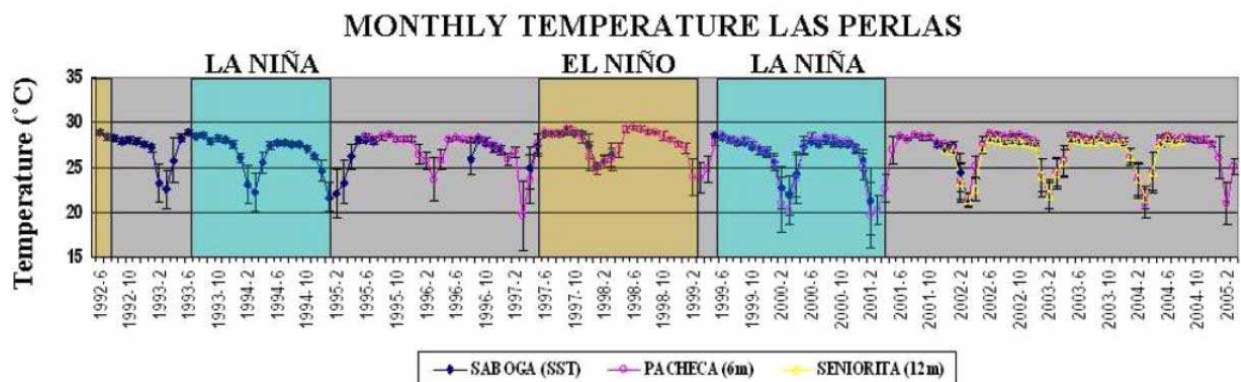
**FIGURE 10.** Cluster analysis based on the degree of polymorphism of the samples (similarity based on Pearson correlation coefficient and UPGMA dendrogram type for each gel) for samples run with primer OPB-7 (analysis for 41 of a total of 46 samples). Each dendrogram presents in each node the error flags and the co-phenetic correlations.

## Discussion

The average density value of *Idanthyrsus cretus* forming the largest reef in the Las Perlas Archipelago (72.2 organisms/10 cm<sup>2</sup>) was similar to that reported by Caline et al. (1988) for *Sabellaria alveolata*. The fauna associated with the *I. cretus* reefs indicated that these polychaete reefs provide, in the SE of Las Perlas Archipelago, a suitable habitat for a diverse fauna in a similar way to that provided by coral reefs in the NW of the archipelago. These assumptions are supported by general observations during the 2004–2005 surveys related to benthic composition in the archipelago (e.g., SE of the archipelago is characterized by uncolonized rock surrounded by sand bottoms while NW is mainly a coral community surrounded by sand bottoms) and by the results of Mair et al. (2009) in which the authors describe a similar grouping for benthic communities in the Las Perlas Archipelago, with a multiscale analysis that shows differences in the benthic communities between SE and NW with respect to species composition and abundance. Hendrick & Foster-Smith (2003), in a description of the *Sabellaria spinulosa* reefs in the UK, quote the official definition of “reef” for the European Union Habitats Directive as follows: “reefs are submarine or exposed at low tide, rocky substrates and biogenic concretions, which arise from the sea floor in the sublittoral zone but may extend into the littoral zone where there is an uninterrupted zonation of plant and animal communities. These reefs generally support a zonation of benthic communities of algae and animal species including concretions, encrustations and corallogenic concretions.” An animal community will use the physical and ecological habitat supplied by any type of reef in the area. In the case of Las Perlas these are coral reefs in the north and west, and sabellariid reefs to the south and east of the archipelago.

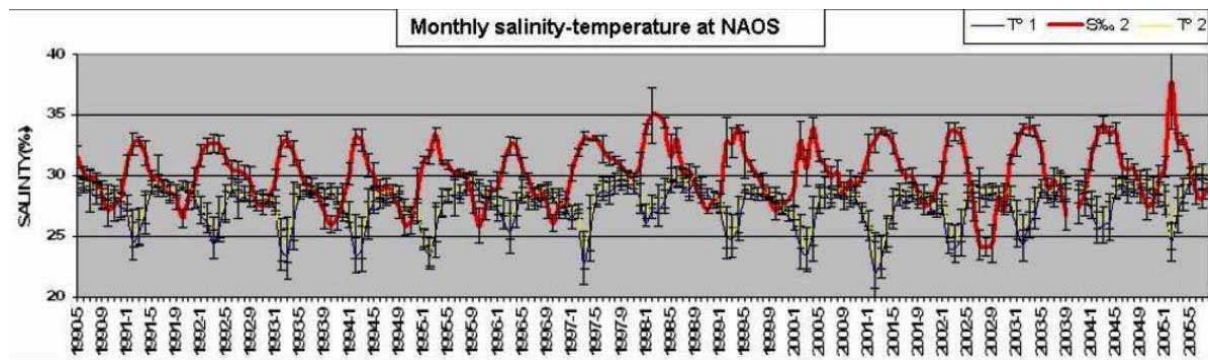
Sea Surface Temperature (SST, Fig. 11) and salinity records (Fig. 12) collected by the Terrestrial and Environmental Science Programme, Smithsonian Tropical Research Institute-STRI illustrated the following:

- An upwelling between January and April of each year that can be identified by a reduction in temperature and increase in salinity. The lowest temperatures are in March and the highest between May and July of each year.
- The mean temperature for the last 15 years (1992–2005) was 26.63°C, but temperatures as low as 14.7°C (Saboga, 22/02/2001) and as high as 30°C (Saboga, 9/11/1997) have been recorded.
- The mean salinity for the last 17 years (1990–2005) in Panama Bay was 30.18‰, but the records show salinities as low as 22‰ (21/10/1995) and as high as 44.8‰ (4/02/2005).



**FIGURE 11.** Sea Surface Temperatures for Las Perlas Archipelago (1992–2005). Data sets were provided by the Smithsonian Tropical Research Institute (STRI 2008).





**FIGURE 12.** Salinity (in red) and temperature (blue and yellow) records for Panama Bay (1990–2005). Data sets were provided by the Smithsonian Tropical Research Institute ([www.stri.org](http://www.stri.org)).

The evidence in the records of this upwelling is a more detailed description of the effect of the Peru Current-PC and the South Equatorial Current-SEC in the area, and gives a better base to the assumptions of a southern origin to the actual larval settlement of sabellariid reefs in Las Perlas Archipelago. A particular aspect of the upwelling in Las Perlas Archipelago is that this phenomenon helps to keep continuous high nutrient and phytoplankton levels in the area (Agujetas-Medrano 2004), creating a high turbidity that causes a low intra-ecosystemic biodiversity for some groups like corals (Diaz et al. 2000). The combination of this low development of corals in the SE area of the archipelago (leading to low competition for colonization space), and the abundance of sediments and hydrodynamics may play a key role in the development of significantly-sized *I. cretus* reefs which are recorded in this study for the first time in the Tropical Easter Pacific.

The ecological information (reef environment and general oceanographic conditions) and the PCA based on data from the five reefs in the SE of Las Perlas Archipelago, plus the two samples collected from a coral reef environment (station 29), confirm that reef building and the distribution of *I. cretus* reefs are dependent firstly on suitable topography, sediment particle size to produce tubes, and wave exposure. In the case of the relationship between sediment particles and the polychaete tubes, the ability to control abdominal growth is important so that the animal is not longer than the tube and vulnerable to predation (Vovelle 1965). In other species (e.g., *Sabellaria aveolata* from the NE Atlantic) there is a link between the size of the building organ, the diameter of the anterior region, and the length of animal, which determines the size of particles used in tube building (Gruet 1984). Coral cover, visibility, and depth are important for the polychaetes individually when they have already settled in a reef environment as they are not able to create their own reef. This was the case for station 29. Thus, the PCA showed two main environments where the sabellariids can be found. The first type of environment is described by PC1 and corresponds mainly to the benthic environments found in the SE of the archipelago (uncolonized rock surrounded by sand bottoms), where the sabellariids are able to develop polychaete reefs due to the higher wave exposure. The second type of environment is described by PC2 and corresponds to the benthic environments found in the NW of the archipelago (coral community surrounded by sand bottoms), where the sabellariids survive only as part of the coral reef community. Therefore, the sabellariids found in station 29 (San Pedro Island, NE of Isla del Rey) may be part of the boundaries between these two environments.

In the case of the molecular techniques, although the cluster analysis with the gels illustrated some differences in the bands between stations, the differences were not statistically significant. In order to identify differences between stations, samples within a particular station should show higher similarity than with samples from other stations. However, the similarity for samples belonging to different stations was of the same order as the similarity within the stations. Therefore no major

differences could be detected in the Las Perlas Archipelago samples. One interpretation of these results is that the different reefs have a similar origin or come from one population. Based on the oceanographic information analyzed (that shows the effect of the Peru Current-PC and the South Equatorial Current-SEC in the area), and the document by Glynn & Mate (1997) that indicates transportation of waters from South America to Panama by these currents, it is feasible to suggest that the original settlement in Las Perlas Archipelago occurred from the south, probably Colombia or Ecuador. The population may have arrived first at San Telmo (the largest reef), where they found good conditions for settlement and survival and, from there, the organisms colonized other sites in Las Perlas.

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